

Enterococci by Membrane Filtration**SM 9230C – 2007**

ADDITIONAL QC REQUIREMENTS FOR THIS METHOD: *Certified or Accredited laboratories using this method are assessed to applicable requirements of SM 9020, 9030, 9040, 9050 and 9060*

Facility Name: _____ LAB ID: _____

Assessor Name: _____ Analyst Name: _____ Inspection Date: _____

Records Examined: SOP Number/Revision/Date: _____ Analyst: _____

Sample ID: _____ Date of Sample Preparation: _____ Date of Analysis: _____

Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
1) Are glass and disposable plastic culture dishes with loose-fitting lids incubated in tightly closed containers?	9222B.1.e				
2) Are opened packages of disposable dishes resealed for storage?	9222B.1.e				
3) Are membrane filters with 0.45 µm pore diameter to provide complete retention of coliform bacteria (usually 0.45 µm) used?	9222B.1.g				
4) Are smooth blunt forceps, without corrugations on the inner sides of the tips, used? Are forceps sterilized before use by dipping in 95% ethyl or absolute methyl alcohol and flaming?	9222B.1.i				
5) Is 10 to 15X magnification and a cool white fluorescent light source used to determine colony counts on membrane filters?	9222B.1.k				
6) Is each lot of purchased or laboratory-prepared medium checked for performance by inoculating it with <i>Enterococcus faecium</i> as a positive control, and <i>Serratia marcescens</i> (a gram-negative species) and <i>Aerococcus viridians</i> (a gram-positive species) as negative controls? Is the use of a heavy inoculum avoided?	9230C.7				
7) Is commercially available medium used (preferably)?	9230C.2.				
mE agar and EIA Substrate (for mE method)					
8) Is 71.2 g of dehydrated mE agar added to 1 L reagent grade water, heated to dissolve, autoclaved at 121°C for 15 min and cooled in a 44 to 46°C water bath?	9230C.2.a				

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9) Is 0.24 g nalidixic acid mixed in 5 mL reagent-grade water, then add a few drops (approx. 0.2 mL) of 0.1N NaOH added to dissolve the nalidixic acid? Is the entire nalidixic acid –NaOH mixture poured through a sterile filter and added to the basal medium?	9230C.2.a				
10) Is 0.15 g 2,3,5-triphenyltetrazolium chloride (TTC) added to agar and mixed well to dissolve?	9230C.2.a				
11) Is the final pH 7.1±0.2?	9230C.2.a				
12) Is 16.5 g of dehydrated EIA substrate added to 1 L of reagent grade water, heated to dissolve, autoclaved at 121°C for 15 min and cooled in a 44 to 46°C water bath?	9230C.2.b				
13) Is the final pH 7.1±0.2?	9230C.2.b				
mEI agar (for mEI method)					
14) Is 0.75 g indoxyl-β-D-glucoside and 71.2 g of dehydrated mE agar added to 1 L reagent grade water, heated to dissolve, autoclaved at 121°C for 15 min and cooled in a 44 to 46°C water bath?	9230C.2.c				
15) Is 0.24 g nalidixic acid mixed in 5 mL reagent grade sterile water; then a few drops of 0.1N NaOH are added to dissolve; and the solution is filter-sterilized and added to the mEI medium?	9230C.2.c				
16) Is 0.02 g 2,3,5-triphenyltetrazolium chloride (TTC) added separately to the mEI medium and mixed?	9230C.2.c				
17) Is the final pH 7.1±0.2?	9230C.2.c.2)				
18) If the larger-scale Nalidixic acid and TTC solutions described in 9230C.2.c.1) & 2) are used in lieu of the smaller-scale media additions described in 9230C.2.c, are the proportions correct?	9230C.2.c.1) and 9230C.2.c.2)				
mEnterococcus agar (for m Enterococcus method)					
19) Is 41.5 g dehydrated mEnterococcus agar added to 1 L reagent grade water and heated to dissolve, <u>not</u> autoclaved?	9230C.2.d.				
20) Is the final pH 7.2±0.2?	9230C.2.d.				

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21) Is fresh medium prepared for each set of samples?	9230C.2.d				
All agar types					
22) Is agar poured into petri dishes to a depth of 4 to 5 mm (approximately 4 to 6 mL) and allowed to solidify?	9230C.2.a, b, c.2) and d				
23) Are all agar plates (<u>EXCEPT</u> mEnterococcus agar) stored in the dark at 2-10°C for no more than 14 days?	9230C.2.a, b, and c.2)				
mE method					
24) Are sample volumes filtered to give 20 to 60 colonies on the membrane surface?	9230C.3.a.1)				
25) Are filters transferred to hardened mE agar in petri dish, avoiding air bubbles beneath the membrane?	9230C.3.a.1)				
26) Are culture dishes inverted and incubated at 41±0.5°C for 48±4 h?	9230C.3.a.2)				
27) After 48±4 h incubation, are filters carefully transferred to EIA medium and incubated at 41±0.5°C for 20 min?	9230C.3.a.3)				
28) Are pink to red enterococci colonies that develop a black or reddish-brown precipitate on the underside of the filter counted?	9230C.3.a.4)				
mEI method					
29) Are sample volumes filtered to give 20 to 60 colonies on the membrane surface?	9230C.3.b.1)				
30) Are filters transferred to hardened mEI agar in petri dish, avoiding air bubbles beneath the membrane?	9230C.3.b.2)				
31) Are culture dishes inverted and incubated at 41±0.5°C for 24±2 h?	9230C.3.b.2)				
32) Are colonies (regardless of color) that are ≥0.5 mm with a blue halo counted as enterococci?	9230C.3.b.3)				
m Enterococcus method					
33) Are sample volumes filtered to give 20 to 60 colonies on the membrane surface?	9230C.3.c.1)				

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34) Are filters transferred to hardened mEnterococcus agar, avoiding air bubbles? Are dishes allowed to stand right side up for 30 min, then inverted and incubated at 35±0.5°C for 48±4 h?	9230C.3.c.2)				
35) Are all light and dark red colonies counted as enterococci?	9230C.3.c.3)				
Calculation of Enterococci Density					
36) Is the density computed from sample quantities producing membrane filter counts within the 20- to 60-colony range? If the colonies are more dense than this, is an estimated number provided or else noted as "too numerous to count" (TNTC) as in Section 9222B.4e?	9230C.4				
37) Are densities recorded as presumptive enterococci per 100 mL?	9230C.4				
Verification Tests					
38) Are the verification steps included in each written enterococci procedure, especially if the results will be used as evidence in a court of law? Does the written procedure include the following steps?	9230C.5				
39) Are enterococci colonies verified by picking selected typical colonies from a membrane and streaking for isolation onto the surface of a BHI agar plate (9230C.2f)? Is BHI Agar incubated at 35±0.5°C for between 24±2 and 48±4 h.	9230C.5				
40) Is a similar-looking portion of a single, well-isolated colony transferred from the BHI agar plate into a BHI broth tube (9230C.2e) and to each of two clean glass slides using a sterile inoculating loop?	9230C.5				
41) Is the BHI broth incubated at 35±0.5°C for 24±2 h?	9230C.5				
42) Are a few drops of freshly prepared 3% hydrogen peroxide added to the smear on one slide? Is the appearance of bubbles interpreted as a positive catalase test and is the indication that the colony is not a member of the <i>Enterococcus</i> genus?	9230C.5				

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43) If the catalase test is negative is a Gram stain made of the second slide? Are fecal streptococci and enterococci interpreted as gram positive, ovoid cells, 0.5 to 1.0 µm in diameter, mostly in pairs or short chains?	9230C.5				
44) Using a sterile inoculating loop, is a loopful of the incubated BHI broth culture transferred to each of the following media and incubated at the following temperatures and times? <ul style="list-style-type: none"> • Bile esculin agar (incubated at 35±0.5°C for 48±4 h); • BHI agar (incubated at 10±0.5°C for 48±4 hrs); • BHI broth (incubated at 45±0.5°C for 48±4 h); and • BHI broth with 6.5% NaCl (incubate at 35±0.5°C for 48±4 h)? 	9230C.5				
45) Does the growth of catalase-negative, gram-positive cocci on bile esculin agar and in BHI broth with 6.5% NaCl broth at 35°C and either in BHI agar at 10±0.5°C or BHI broth at 45±0.5°C confirm that the colony belongs to the <i>Enterococcus</i> genus?	9230C.5				

Notes/Comments